that the invention of claim 48 is considered by the Examiner to be independent and distinct from the invention of claims 30-47. By the present amendment, claim 48 has been canceled.

III. Objection to Figures 6A-6B Under 35 USC 132

The drawing sheet submitted February 11, 2000 (Figs. 6A-6B) was objected to as allegedly being new matter. The drawing sheet had been submitted in response to an objection under 37 CFR 1.83(a) for not showing "every feature of the invention specified in the claims" (Office action dated August 11, 1999).

According to 37 CFR 1.83(a), "The drawing in a nonprovisional application must show every feature of the invention specified in the claims. However, conventional features disclosed in the description and claims, where their detailed illustration is not essential for a proper understanding of the invention, should be illustrated in the drawing in the form of a graphical drawing symbol or a labeled representation (e.g., a labeled rectangular box)." Furthermore, only details of "sufficient importance" need be shown (see MPEP 608.02(d)).

A new drawing can be added by amendment provided that it does not introduce new matter (35 USC 132). There is no new matter if the added subject matter is supported by the original disclosure in accordance with the first paragraph of 35 USC 112, that is, if the original disclosure conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, the applicant was in possession of the invention (e.g., Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 19 USPQ2d 1111, Fed. Cir. 1991).

Pending claim 30 recites: "An instrument for monitoring a nucleic acid amplification reaction over multiple thermal cycles, comprising:

(a) a thermal cycler capable of alternately heating and cooling, and adapted to receive, a reaction vessel containing an amplification reaction mixture comprising a target nucleic acid, reagents for nucleic acid amplification, and a detectable nucleic acid binding agent, in a sealed vessel condition; and

(b) an optical system including a detector operable to detect an optical signal related to the amount of amplified nucleic acid in the reaction vessel over a multiple-cycle period, with the reaction vessel in a sealed condition, allowing determination of a cycle-dependent change in such optical signal over a multiple-cycle period with the reaction vessel in its sealed condition.

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Various embodiments and features of the claimed invention are illustrated in the specification at page 13 line 27 through page 15 line 13 and Example VIII (pages 28-30), for example. On page 14, beginning at line 10, reference is made to a thermocycler (also known as a thermal cycler) which contains a heat block capable of carrying out 48 amplification reactions simultaneously, as was available from Perkin-Elmer Cetus Instruments at the time of filing (see attached photograph from a Perkin Elmer Cetus catalog from Summer, 1990). On page 11, the catalog notes that the cited cycler was "microprocessor-controlled", to implement a user-selected cycling profile. This is demonstrated in Example VIII, wherein such a thermocycler was used to automatically cycle between 94°C and 50°C for 1 minute each, for 30 cycles.

The presently claimed invention also includes an optical system that can be used to determine a cycle-dependent change in an optical signal from an amplification reaction over a multiple-cycle period, while the reaction vessel remains in a sealed (unopened) condition (e.g., page 14 lines 12-16). In one embodiment, fiber optic leads can be placed in proximity to each of one or more reaction vessels, so that an optical signal can be transmitted from each reaction mixture to a detector. Such a fiber optic lead can be positioned in proximity to a reaction mixture, without an intervening vessel wall, as noted at page 14, lines 26-28. According to another embodiment, each reaction vessel can have a clear or translucent portion (e.g., cap) through which an optical signal can be collected. In yet another embodiment, the specification notes that an optic fiber is not required where a detector and thermocycler are housed together, rather than independently (see page 14 lines 31-34, for example). Example VIII refers to an optical detection system that included a Spex-Fluorolog-2 fluorometer equipped with a fiber optic accessory and standard data collection software (see page 28 lines 28-30). Other detection formats are also discussed, such as a microtiter format (page 14 line 35 et seq.), and detection by optical density-based methods and light scattering (page 15, lines 7-25).

In the Amendment submitted February 11, 2000, pages 13 and 14 of the specification were amended to include reference numbers for exemplary components in order to show congruence with Figs. 6A and 6B (see Amendment of 2-11-00 at pages 1-2). From the foregoing, it is respectfully submitted that Figs. 6A and 6B do not constitute new matter since they are supported by the original disclosure in a manner sufficient to convey with reasonable clarity to those skilled in the art that, as of the filing date sought, the applicant was in possession of the invention.

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IV. Obviousness Rejection Under 35 U.S.C. 103(a)

Claims 30-47 were rejected as allegedly being obvious in view of Haff et al. (Amplifications 1:8-10, 1989) in view of Mackay (EP 266881). The rejection is respectfully traversed.

The PTO has the burden of establishing *prima facie* obviousness, and can meet this burden "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references" In re Fine, 837 F.2d 1071, 5 USPQd2 1596 (Fed. Cir. 1988).

Haff et al. teaches an approach very different from the present invention. In Haff et al., several identical PCR reaction mixtures (100 µL each) were run in parallel for up to 25 PCR thermal cycles. After a selected number of cycles (4, 8, 12, 14, 16, 18, 20 and 25 -- see Fig. 1), a 25 µL aliquot was withdrawn manually by pipet from each mixture. To each aliquot was added 200 µL of dye solution (Hoechst 33258, 2 µg/mL after 1:500 dilution of 1 mg/mL stock solution) and then TE buffer to make a final assay volume of 2.0 mL (see third paragraph under "Method" on page 9). *Thus, there was no detection dye in the reaction mixtures during amplification. Rather, dye was added to aliquots from samples only after a selected number of thermal cycles had been completed.*

Mackay teaches an apparatus for measuring sample components using fibre optics (see Abstract). Temperature control is not discussed. There is no suggestion of utilizing a thermal cycler capable of iteratively heating and cooling a sample to promote amplification of target nucleic acids, nor of detecting an increase in signal over multiple cycles while the reaction vessel remains in a sealed condition.

There is nothing that would have motivated one of skill in the art to modify the cited references to make the presently claimed invention.

In particular, the approach in Haff et al. is deficient for at least several reasons. First, Haff et al. teaches running replicates of a reaction mixture to allow different aliquots to be removed after different numbers of cycles. This approach is susceptible to the risk of variability among different replicates and clearly is less desirable than conducting all measurements on a single reaction mixture in a sealed condition. Second, Haff et al. failed to recognize that an indicator reagent could be included in a nucleic acid reaction mixture to allow amplification to be measured over multiple cycles without opening the reaction vessel.

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Furthermore, although the Haff article mentions the possibility of automating his method on page 9 under "Instrumentation", the cited device (a Perkin-Elmer Model LS-2B spectrophotometer with an autosampler) would only have automated the transfer of each assay mixture (the result of mixing an aliquot of a PCR reaction mixture with dye solution and TE buffer as discussed in paragraph 5 above) from an autosampling tray into and out of the fluorescence cuvette for fluorescence measurement. The cited autosampler had no capacity to automate the collection of aliquots from the PCR reactions, nor mixing of those aliquots with dye solution, so these steps would still need to be done by the user.

In essence, Haff et al. teaches away from the present invention.

Nor are the deficiencies of the Haff et al. remedied by Mackay given the absence of any suggestion of combining a detector with a thermal cycler, or any guidance on how nucleic acid amplification could be followed over multiple cycles in a single reaction mixture maintained in a sealed condition.

Since the cited references fail to suggest that the claimed invention should be made, withdrawal of the rejection is respectfully requested.

V. Double Patenting Rejection

The claims were rejected for alleged obviousness-type double patenting over claims in copending application Ser. No. 08/266,061. It is requested that this rejection be held in abeyance until patentable subject has been established.

Appendix Showing Markup of Changes

Added text is indicated by bold and underlining.

Paragraph at page 2 lines 32-38:

Copending U.S. Serial No. 563,758, filed August 6, 1990 **(now U.S. Patent No. 5,210,015)**, and incorporated herein by reference, describes an alterative assay method for detecting amplified nucleic acids. The process employs the 5' to 3' nuclease activity of a nucleic acid polymerase to cleave annealed, labeled oligonucleotides from hybridized duplexes and release labeled oligonucleotide fragments for detection. The method is suitable for detecting PCR products and requires a primer pair and a labeled oligonucleotide probe having a blocked 3'-OH terminus to prevent extension by the polymerase.

Paragraph at page 9 line 31 to page 10 line 5:

In the disclosed embodiment, Taq DNA polymerase is preferred although this is not an essential aspect of the invention. Taq polymerase, a thermostable polymerase, is active at high temperatures. Methods for the preparation of Taq are disclosed in U.S. Patent No. 4,889,818 and incorporated herein by reference. Taq polymerase is available from Perkin Elmer Cetus Instruments (PECI). However, other thermostable DNA polymerases isolated from other Thermus species or non Thermus species(e.g., Thermus thermophilus or Thermotoga maritima), as well as non-thermostable DNA polymerase such as T4 DNA polymerase, T7 DNA polymerase, E. coli DNA polymerase I, or the Klenow fragment of E. coli, can also be used in PCR. Methods for providing thermostable DNA polymerases are provided in copending Serial Nos. 455,967, filed December 22, 1989 **(now U.S. Patent No. 5,618,711)**; 567,244, filed August 13, 1990 **(now U.S. Patent No. 5,374,553)**; and 590,213, 590,466 **(now U.S. Patent No. 5,455,170)**, and 590,490, filed September 28, 1990, which are all incorporated herein by reference.

Paragraph at page 13 lines 8-16:

Methods for reverse transcribing RNA into cDNA are well known and described in Maniatis et al., supra. Alteratively, preferred methods for reverse transcription utilize thermoactive DNA polymerases. These methods are described in commonly assigned, copending, U.S. Serial No. 455,611, filed December 22, 1989 **(now U.S. Patent No. 5,322,770)**, and incorporated herein by reference. U.S. Serial No. 455,611 describes a procedure for coupled reverse transcription/amplification of an RNA template using a thermostable DNA polymerase. The present specification teaches that intercalating agents do not prevent DNA polymerase activity. Consequently, the present method provides a homogeneous detection assay for RNA targets as well as DNA targets.

Paragraph page 15 lines 14-25:

> In another embodiment of the invention, following amplification, the size of the amplified product is determined without the use of a probe or size fractionation methods such as HPLC or gel electrophoresis. Copending U.S. Serial No. 601,840, filed October 23, 1990 **(now U.S. Patent No. 5,269,937)**, which is incorporated herein by reference, describes a method for determining the average molecular weight of a PCR product using light scattering. The method is suitable for use in conjunction with the present invention especially when the homogeneous assay result is detected using a spectra fluorometer. A fluorometer reads emissions at the fluorescence wavelength, according to the present invention, and measures light scattering, for example, at a 180° angle. This aspect of the invention is particularly useful for determining if amplification has occurred and simultaneously distinguishing the amplified target product from, for example, primer-dimer and high molecular weight DNA.

Paragraph at page 17 lines 7-14:

> In general, it is preferred but not essential that the DNA polymerase is added to the PCR reaction mixture after both the primer and template are added. Alteratively, for example, the enzyme and primer are added last or the PCR buffer or template plus buffer are added last. It is generally desirable that at least one component that is essential for polymerization not be present until such time as the primer and template are both present, and the enzyme can bind to and extend the desired primer/template substrate (see U.S. patent application Serial No. 481,501, filed February 16, 1990, **now U.S. Patent No. 5,411,876,** which is incorporated herein by reference).

Paragraph page 18 lines 31-37:

> Methods for quantitating nucleic acids are described in commonly assigned, copending U.S. Serial Nos. 254,889, filed October 7, 1988 **(now U.S. Patent No. 5,389,512)**, and 413,623, filed September 28, 1989 **(now U.S. Patent No. 5,219,727)**. These applications are incorporated herein by reference. These applications describe PCR-based methods using an internal standard to determine either the relative amount of a target or accurately quantitate the amount of target present prior to amplification, respectively. The present invention is suitable in conjunction with the methods described in the '889 and '623 applications.